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EFFECTS OF PERUVIAN MAIZE EXTRACTS ON GROWTH, DEVELOPMENT, AND FECUNDITY OF THE EUROPEAN CORN BORER

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Abstract—Twelve Peruvian maize, *Zea mays*, accessions were selected because of their relatively high level of field resistance to first-generation European corn borer (ECB), *Ostrinia nubilalis*, larval leaf-feeding. Water extracts of freeze-dried, powdered, leaf tissue were incorporated into a standard ECB diet, fed to larvae, and the effects on larval growth, development, and fecundity were measured. Larval and pupal weights were monitored as were the time elapsed in the larval, pupal, and adult stages. Adult fecundity and egg fertility were recorded. The experiment was a randomized block design (larvae and pupae) or a completely randomized design (adults) and analyzed with ANOVA ($\alpha = 0.05$). Pairwise comparisons were made between groups of insects grown on diets containing extracts from the Peruvian lines, a standard diet, or diets containing extracts of a known susceptible inbred, and a known resistant inbred line. Survival was analyzed with a chi-squared test ($\alpha = 0.05$). Two Peruvian accessions significantly reduced female larval and pupal weights, extended pupal and adult development time, and decreased survival of pupae and adults. Water extracts also had a pronounced impact on males; two accessions significantly reduced pupal weight and extended the time required to pupate, and one

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reduced male survival to adults. The results indicate that water-soluble factors from resistant Peruvian accessions inhibit the growth, developmental time, and survival of ECB. These resistance factors could be useful in the development of maize germplasm with insect-resistant traits.

Key Words—European corn borer, *Ostrinia nubilalis* (Hübner), growth, development, fecundity, oviposition, behavior, eggs, DIMBOA, water extract, plant extract, maize, Peruvian maize, maize accessions, host plant resistance.

INTRODUCTION

The European corn borer, *Ostrinia nubilalis* (Hübner) (ECB) is a significant pest of maize, *Zea mays* L., and other crops in the United States (Mason et al., 1996). One approach to reduce yield losses caused by ECB is to develop inbred lines of maize with natural physical and/or chemical resistance that can be used as parents of hybrids utilized by farmers (Guthrie and Barry, 1989). New sources of maize resistance to ECB have been identified recently by field evaluating Peruvian maize from the extensive collections of germplasm at the United States Department of Agriculture North Central Regional Plant Introduction Station (NCRPIS) (Abel et al., 1995). These exotic accessions have proven to be a rich resource for germplasm with new types of resistance against ECB and other maize pests (Davis et al., 1988; Wilson et al., 1995).

Many resistance mechanisms in plants result from the production and accumulation of chemicals that repel or deter insect herbivores (nonpreference), or these chemicals may inhibit growth and development (antibiosis) of those insects that feed on the plant tissue (Maxwell and Jennings, 1980; Panda and Khush, 1995). A well-studied example is the protection some maize genotypes have against ECB larvae because they synthesize hydroxamic acids such as DIMBOA [2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one] and related compounds (Klun et al., 1970). Leaf concentrations of DIMBOA decrease as the plants develop in all DIMBOA-related maize genotypes studied thus far, so this resistance mechanism fails to protect plants from ECB attack throughout the growing season in the United States. New genotypes are continually being created by plant breeders and entomologists to improve yields, generate high-quality agronomic traits, and maintain resistance to pests over the entire field season. Newly discovered maize germplasm with resistance to ECB and other pests may have novel, as yet undiscovered, biochemical pathways that could serve a significant role in the plant's protection.

Peruvian maize collections maintained at the NCRPIS extend genetic diversity beyond the uniformity of lines adapted solely to North America and thus provide reserves of seed with insect-resistant traits. More than 1600 Peruvian genotypes were evaluated for first-generation ECB resistance, and 11 entries showed promise as a new genetic reservoir of insect resistance (Abel et al., 1995; Wilson et al., 1995). Chemical evaluation of the Peruvian maize leaf tissue revealed that the amounts of DIMBOA present were not high enough to account for the ECB leaf-feeding resistance. Identification of a new chemical resistance mechanism would be an important step toward developing maize germplasm that is resistant to ECB feeding. The objective of the present study was to evaluate whether ECB resistance in the Peruvian accessions observed by Abel et al. (1995) is caused by chemical factors and to determine the genotypes that contain defensive chemicals at sufficient levels to cause inhibition of growth, development, and fecundity in ECB.

METHODS AND MATERIALS

Extraction of Plant Material. Eleven Peruvian lines of maize (Ames 10623, PI 503720, PI 503722, PI 503723, PI 503725, PI 503727, PI 503728, PI 503731, PI 503764, PI 503806, and PI 503849), which were field tested and found resistant to ECB larval leaf-feeding by Abel et al. (1995), were selected for study along with a resistant line PI 485320 (Abel and Wilson, unpublished observations), a susceptible check (WF9), and a resistant check (CI31 A) (Table 1). All accessions were planted at the Plant Introduction Station at Ames, Iowa, on May 15, 1995. Leaves for chemical extraction were prepared following the method of Wilson and Wissink (1986). When the maize reached the V7–V8 leaf stage (Ritchie et al., 1986) (June 28, 1995), ca. 40 plants of each accession were cut off ca. 15 cm below the whorl, packaged by accession, and frozen immediately at −20°C. Leaves were removed from refrigeration and freeze dried in a LABCONCO model 5 freeze dryer (Kansas City, Missouri), milled to a fine powder by using a Thomas Wiley Laboratory Mill, model No. 4 (Philadelphia, Pennsylvania), and stored again at −20°C in 0.5-liter glass jars until extraction. Later, powdered leaf material of each accession (20.25 g) was extracted with 500 ml of distilled water in a commercial Waring Blender (model 36BL22, New Hartford, Connecticut); after filtration (Whatman No. 1, Whatman International, Maidstone, Kent, England) the liquid extracts were frozen at −20°C and lyophilized to reduce the volume in a LABCONCO FreeZone R 18 Liter Freeze Dry System model 77550-10. The remaining residues were stored at −20°C until use in the growth, development, and fecundity studies (Table 1).

Insects. Laboratory-reared ECB larvae that were ca. eight generations removed from the field and reared according to the methods of Reed et al. (1972) were used in this study. Adults eclosed in specially designed cages (58.7 × 58.7 × 63.7 cm) made of angle-and-strap aluminum frame (1.9 and 2.3 cm, respectively) covered on the sides and bottom with 16 × 18 mesh brass cloth (Binder and Robbins, 1996). The top of the cage was covered with 5 × 5 mesh galva-

TABLE 1. ECB LARVAL RESISTANCE RATINGS FOR PERUVIAN MAIZE ACCESSIONS AND AMOUNT OF WATER-EXTRACTABLE RESIDUE INCORPORATED INTO 200 GRAMS OF ARTIFICIAL DIET

Accession	Mean leaf-feeding ratings[a]	Extract weight (g)[b]	Peruvian maize race
WF9	7.1 a	3.0	Susceptible check
PI 503725	3.2 de	3.4	Mochero
PI 503806	3.2 de	3.0	Alazan
PI 503849	3.2 de	3.2	Alazan
PI 503764	3.0 def	3.6	Mochero
Ames-10623	3.0 def	3.9	Arizona
PI 503720	2.9 def	3.3	Mochero
PI 503722	2.9 def	3.7	Mochero
PI 503728	2.9 def	3.7	Mochero
PI 503727	2.8 ef	3.2	Mochero
PI 503723	2.6 efg	2.6	Mochero
PI 503731	2.2 fg	3.2	Mochero
CI31A	1.8 g	3.0	Resistant check
PI 485320	4.0	3.3	Mochero

[a]Means followed by the same letter are not significantly different according to the LSD test (P = 0.05) (N = 8). Visual ECB leaf feeding ratings on manually infested plants (1–9 scale; Guthrie et al., 1960). These ratings were taken by Abel et al. (1995) during the 1992 growing season at the USDA Plant Introduction Station at Ames, Iowa (except PI 485320, which was completed on a later date and therefore not included in the analysis).
[b]Extracted from 20.25 g of powdered dried leaf.

nized hardware cloth, which permitted oviposition on the underside of wax paper sheets positioned on top of the cage. Two feeding stations were included in each cage. One was a cotton pad suspended from a brass rod 19.5 cm from the top of the cage and moistened daily with water. The other, a molded plastic unit (10.3 cm^2) with 16 wells (each with a capacity of 1 ml), was located on the bottom of the cage and its wells were filled with 1.4% (w/v) agar gel containing 39.4% (w/v) sucrose (Leahy and Andow, 1994). Egg masses were removed from the waxed sheets and placed in 70-ml screw-capped glass bottles. The bottles were incubated at 27°C, 75–80% relative humidity, and with continuous light until larvae hatched. ECB larval neonates within 12 hr of hatch were used in all tests.

Diet Formulation with Maize Extracts. Standard ECB diet was prepared according to Reed et al. (1972). Each Peruvian maize, WF9, and CI31A dry extract was dissolved in 20 ml distilled water and mixed into a 200-ml aliquot hot liquid ECB diet (50–56°C). Control diet consisted of 200 ml of standard ECB diet plus 20 ml of distilled water. Each diet was poured into Petri dishes to cool and harden overnight at room temperature. A total of 15 diets were prepared and stored for ca. seven nights at 4.4°C until the experiment was started. Diets were warmed to room temperature before use.

ECB Larval Growth and Development Studies. Three neonate larvae were transferred to each six-dram vial with experimental diet plugs (1.5 cm diameter × 1.2 cm deep, 4 g), and the vial was tightly plugged with a piece of autoclaved cotton (nonabsorbent, nonsterile cotton, Acco, Valley Park, Missouri). Vials were incubated at 27° ± 2°C, relative humidity of 75 ± 5%, with continuous light, in a Percival Incubator (model CE-2, Boone, Iowa). After six days, the two smaller larvae were removed, leaving the most robust individual in each vial. Larval weights were measured after 13 days (±0.1 mg) on a Mettler AE 100 Electronic Balance (Hightown, New Jersey). The balance interfaced with an IBM PS 2 computer (International Business Machines Corp., Armonk, New York) to record larval weight automatically in QuickBasic 1.1 (Microsoft Corp., Redmond, Washington). Data were transferred to Microsoft Excel 5.0 for tabulation. On the day following pupation, each ECB was removed from the vial, weighted (±0.1 mg) on an Ohaus GA 200 D balance (Florham Park, New Jersey), and transferred to a 33-cc plastic cup (covered with a plastic snap-on lid) to monitor for adult eclosion.

ECB Oviposition Studies. Each adult female was paired with a male from the general colony in a 14 × 18 mesh brass cloth cylindrical mating cage, ca. 8 cm height × 8 cm diameter, with a brass cloth bottom and a 4 mesh galvanized hardware top (Kira et al., 1969). The top was covered with a glass petri dish, which served as an oviposition substrate. Cages were kept in an incubation chamber at 16L:8D; 24.5 (day):18.5 (night) ± 2.5°C; 85 ± 10% relative humidity. Optimum humidity was maintained by placing the cages on water-soaked cotton (Acco sterile absorbent cotton). Following mating, female ECB moved to the top of the cage and deposited egg masses on the underside of the Petri dish. Each female was considered successfully mated if she produced fertile eggs. Petri dishes were changed each day; the fecundity (number and size of egg masses for each female) was monitored by measuring each egg mass with a digital analyzer (Decagon Industries Monochrome AgVision Imaging System, Pullman, Washington) (Binder and Robbins, 1996). Petri dishes with egg masses were incubated under continuous light at 24.5 ± 1.5°C and 96 ± 2% relative humidity to facilitate optimum larval hatch. Fertility or the percentage of viable eggs (= hatched larvae) was determined by visual examination under a 10× dissecting microscope (model 41, American Optical, Buffalo, New York).

Male adults from the test diets were not used in the mating portion of the experiment because female mating, longevity, and oviposition were the foci of the study.

Statistical Design and Analysis. We used a randomized block design with 15 replicates where an experimental unit consisted of two vials with one larva per vial. Male and female data were analyzed separately. Mortality and differences in sexes in each experimental unit resulted in incomplete data. Treatment means in the randomized block analysis were adjusted for missing data, and significance tests were adjusted for all pairs of adjusted means. PROC GLM and LSMEANS (SAS Institute, Inc., Cary, North Carolina; www.sas.com) were used for computing the analysis. Differences from expected values for survival to pupae and adults were analyzed with a chi-square test using PROC FREQ (SAS Institute, Inc.). Adult longevity, fecundity, and fertility were analyzed in a completely randomized design with PROC GLM.

RESULTS

Incorporation of water-extractable compounds from dried leaf tissue of some Peruvian accessions and CI31A caused reductions in ECB larval and pupal weights, delays in development, and reduced fecundity as compared with larvae reared on WF9 or the standard diet (Tables 2–4). ANOVA and chi-square evaluations indicated significant differences for all female larval and adult variables except fertility. Males also were affected by Peruvian maize extracts; pupal weight, pupal development time, and survival to adults were affected. The data show that there were no differences between treatments in percentage of larvae of either sex surviving to the pupal stage, and, therefore, Peruvian extracts impaired development of ECB primarily in the pupal and adult stages.

Of the 12 Peruvian lines tested, water extracts of PI 485320 and PI 503723 had the most deleterious effect on female ECB larval weight and development but no effect on male larval weight (Table 2). Female 13-day larval weights for individuals reared on PI 485320 and PI 503723 diets were lower than those reared on the WF9 diet, but their weights were not different from those reared on the resistant check, CI31A. Female larvae that were reared on diet incorporating water extracts of the remaining accessions were not different in weight from those reared on standard diet (Table 2).

Measurement of pupal weight was a reliable indicator of ECB performance because, compared to larval weight, it was less variable. The stability of the pupal weight proved useful to gauge differences in ECB performance. A comparison of pupal weight, therefore, was used to evaluate differences in the effects of extracts of Peruvian accessions. Mean pupal weight of females reared on diets containing water extracts of accessions PI 503723 and PI 503720 were lower than those reared on standard diet or diet containing water extracts of WF9 and PI 503806, but not different from females reared on diet with water extract of CI31A (Table 2). Mean pupal weight of females reared on PI 485320 was the lowest of all treatments tested and even lower than mean pupal weight of females reared on the resistant check CI31A (Table 2). Mean pupal weight of males reared on diets containing water extracts of Peruvian accessions PI 503723 and PI 485320 were significantly lower than those reared on WF9 diet but not significantly different from males reared on CI31A (Table 2).

TABLE 2. LARVAL AND PUPAL WEIGHTS OF ECB REARED ON DIETS CONTAINING WATER EXTRACTS OF PERUVIAN MAIZE ACCESSIONS, INBREDS WF9 AND CI31A, AND STANDARD CONTROL DIET[a]

	Thirteen-day larval weight (mg) ± SE				Pupal weight (mg) ± SE			
Treatment	Male	*N*	Female	*N*	Male	*N*	Female	*N*
PI 503806	82.9 ± 3.6 a	14	120.9 ± 4.9 a	15	69.2 ± 2.9 a	12	96.6 ± 3.6 a	15
WF9 (susceptible check)	85.0 ± 4.0 a	11	115.0 ± 4.8 a	19	72.3 ± 3.3 a	10	95.1 ± 3.5 a	19
PI 503731	85.1 ± 3.9 a	10	113.3 ± 4.6 a	20	67.4 ± 3.2 ab	10	92.3 ± 3.4 a	20
Standard Diet	86.5 ± 3.6 a	12	116.3 ± 4.8 a	15	69.0 ± 2.9 a	12	91.7 ± 3.5 a	15
Ames 10623	83.0 ± 3.9 a	10	114.4 ± 5.2 a	17	73.5 ± 3.2 a	10	91.3 ± 3.8 a	17
PI 503727	86.6 ± 4.0 a	9	111.4 ± 5.1 a	17	70.3 ± 3.2 a	9	90.2 ± 3.7 a	17
PI 503722	85.3 ± 3.8 a	10	113.5 ± 4.4 a	20	66.1 ± 3.1 abc	10	89.8 ± 3.2 a	20
PI 503849	79.3 ± 3.6 a	12	107.9 ± 4.8 ab	16	70.2 ± 2.9 a	12	87.6 ± 3.5 ab	16
PI 503764	78.2 ± 3.3 a	16	106.4 ± 5.1 ab	13	60.2 ± 2.7 abc	16	85.0 ± 3.7 ab	13
PI 503725	82.9 ± 3.7 a	11	111.0 ± 4.7 a	19	65.6 ± 3.0 abc	11	83.2 ± 3.4 ab	19
PI 503728	78.2 ± 3.4 a	13	97.7 ± 4.7 abc	16	63.1 ± 2.8 abc	13	77.7 ± 3.4 abc	16
PI 503720	76.2 ± 4.0 a	10	96.4 ± 4.6 abc	19	61.2 ± 3.2 abc	10	71.3 ± 3.4 bc	19
PI 503723	69.5 ± 4.0 a	9	87.7 ± 4.5 bc	20	50.4 ± 3.3 c	9	68.8 ± 3.3 bc	20
CI31A (resistant check)	74.9 ± 4.1 a	10	90.6 ± 4.6 bc	19	57.7 ± 3.3 abc	10	64.3 ± 3.4 c	19
PI 485320	73.5 ± 4.0 a	10	81.5 ± 4.6 c	19	52.1 ± 3.2 bc	10	54.2 ± 3.4 d	19

[a]Thirteen-day larval and pupal weights are expressed as the means (± SE). Each category was analyzed with ANOVA ($\alpha = 0.05$), and pairwise comparisons within each category were made with an LSMEANS test ($\alpha = 0.05$). Values in each category followed by the same letter indicate means that were not significantly different.

Addition of water extracts of some Peruvian accessions to diets extended larval development time, pupal development time, and the overall time required to reach adult stage (Table 3). Extracts of accessions PI 503723 and PI 485320 extended overall development time for females as compared to females reared on standard diet or on diet with extract from leaves of the susceptible check WF9 (Table 3). Females reared on diet with extracts of either PI 503723 or PI 485320 had overall development times that were not significantly different from females reared on diets with extracts of leaves of the resistant check CI31A (Table 3). Extracts of some Peruvian accessions also shortened adult longevity for females as compared to females reared on standard diet or on diet with extract from leaves of the susceptible check WF9 (Table 4).

Water extracts of Peruvian accessions had a pronounced impact on male development times (Table 3). The extract of PI 503723 and PI 485320 extended male larval development time when compared with males reared on standard diet. There was no difference among diets with extracts of Peruvian or WF9 leaf for male pupal development time or in the overall time for development to the adult stage (Table 3).

ECB survival to the pupal stage, 86.7% or greater on all diets tested, was not affected by the type of accession (Table 4). Survival from the pupal to the adult stage, however, was affected by the type of accession in both males and females (Table 4). When the chi-square test for male survival to adults was analyzed without the PI 485320 group, differences among males reared on the remaining 14 treatments were eliminated. This test thus showed that PI 485320 was responsible for the observed differences in male survival from the pupal to the adult stage. Female survival from pupae to adults, in contrast, separated into two groups that were different. Females reared on diets containing extracts of PI 503720, PI 505723, CI31A, and PI 485320 were not different from each other in survival to adults but were different from females of the other 11 treatments, which also were not different in survival among themselves (Table 4).

There was an effect of Peruvian maize extracts on fecundity of surviving females (Table 4). No differences were observed in egg fertility among females reared on diets with different Peruvian maize extracts. A high percentage of viable egg masses produced by females grown on diets incorporating extracts shows that the extracts do not affect the female's offspring.

DISCUSSION

Water-soluble compounds from freeze-dried Peruvian maize accessions PI 485320, PI 503720, and PI 503723 had an effect on ECB growth and development. Water extracts of the remaining field-tested insect-resistant accessions had no effect in the laboratory. Lack of activity of some extracts could have resulted from

TABLE 3. LARVAL AND PUPAL DEVELOPMENT TIMES[a] OF ECB WHEN REARED ON DIETS CONTAINING WATER EXTRACTS OF PERUVIAN MAIZE ACCESSIONS, INBREDS WF9 AND CI31A, AND A STANDARD CONTROL DIET

Treatment	Larval development time (days)		Pupal development time (days)		Overall larval and pupal development time (days)	
	Male	Female	Male	Female	Male	Female
PI 503806	15.3 ± 0.3 ab	15.4 ± 0.4 a	7.1 ± 0.3 ab	6.5 ± 0.2 abc	22.4 ± 0.4 a	22.3 ± 0.5 a
WF9 (susc. chk)	15.4 ± 0.4 ab	16.1 ± 0.3 ab	7.6 ± 0.3 ab	6.1 ± 0.2 a	23.2 ± 0.4 ab	22.3 ± 0.4 a
PI 503731	15.1 ± 0.3 ab	15.8 ± 0.3 ab	7.2 ± 0.3 ab	6.4 ± 0.2 ab	22.6 ± 0.5 ab	22.3 ± 0.4 a
Standard Diet	15.0 ± 0.3 a	15.7 ± 0.3 ab	7.5 ± 0.3 ab	6.3 ± 0.2 a	22.9 ± 0.5 ab	22.2 ± 0.4 a
Ames 10623	15.0 ± 0.3 a	16.0 ± 0.4 ab	7.0 ± 0.3 ab	6.2 ± 0.2 a	22.1 ± 0.4 a	22.3 ± 0.5 ab
PI 503727	15.7 ± 0.4 ab	16.7 ± 0.4 ab	6.6 ± 0.4 a	6.4 ± 0.2 ab	22.0 ± 0.6 a	23.1 ± 0.4 ab
PI 503722	15.3 ± 0.3 ab	15.9 ± 0.3 ab	6.9 ± 0.3 a	6.6 ± 0.2 abc	21.9 ± 0.5 a	22.8 ± 0.4 ab
PI 503849	15.4 ± 0.3 ab	15.9 ± 0.3 ab	7.7 ± 0.3 ab	6.5 ± 0.2 ab	23.1 ± 0.4 ab	22.7 ± 0.4 ab
PI 503764	15.8 ± 0.3 ab	16.6 ± 0.4 ab	7.1 ± 0.3 ab	6.6 ± 0.2 abc	23.1 ± 0.4 ab	23.1 ± 0.4 ab
PI 503725	15.5 ± 0.3 ab	16.5 ± 0.3 ab	7.4 ± 0.3 ab	6.7 ± 0.2 abc	23.0 ± 0.4 ab	22.7 ± 0.4 ab
PI 503728	15.4 ± 0.3 ab	16.2 ± 0.3 ab	7.4 ± 0.3 ab	6.6 ± 0.2 abc	23.0 ± 0.4 ab	23.0 ± 0.5 ab
PI 503720	16.0 ± 0.3 ab	16.7 ± 0.3 ab	7.0 ± 0.3 ab	6.9 ± 0.2 abc	23.0 ± 0.4 ab	23.8 ± 0.5 abc
PI 503723	16.7 ± 0.4 b	17.7 ± 0.3 c	7.2 ± 0.4 ab	7.1 ± 0.2 abc	23.9 ± 0.6 ab	24.9 ± 0.5 bc
CI31A (res. chk)	17.0 ± 0.4 b	17.8 ± 0.3 c	9.2 ± 0.6 b	7.7 ± 0.3 c	25.2 ± 0.7 b	26.1 ± 0.5 c
PI 485320	16.7 ± 0.4 b	18.7 ± 0.3 d	8.5 ± 0.7 ab	7.6 ± 0.3 bc	25.2 ± 1.1 ab	25.5 ± 0.6 c

[a]Male and female larval development time, pupal development time, and overall larval and pupal development time are expressed as the means (±SE). Each category was analyzed by ANOVA ($\alpha = 0.05$), and pairwise comparisons within each category were made with an LSMEANS test ($\alpha = 0.05$). Values in each category followed by the same letter indicate means that were not significantly different.

TABLE 4. SURVIVAL OF MALES AND FEMALES, FEMALE LONGEVITY AND FECUNDITY, AND EGG FERTILITY OF ECB REARED ON DIETS CONTAINING EXTRACTS OF PERUVIAN MAIZE ACCESSIONS, INBRED WF9 AND CI31A, AND STANDARD CONTROL DIET[a]

Treatment	Survival of both sexes to the pupal stage [% (*N*)]	Survival from pupal to the adult stages [% (*N*)] Male	Survival from pupal to the adult stages [% (*N*)] Female	Female longevity (days)	Female fecundity (eggs/female)	Fertility (% viable eggs/female)
PI 503806	90.0(27)	83.3(10)a	86.7(12)a	12.3 ± 1.04 a	656.4 ± 36.7 a	91.4 ± 8.3
WF 9 (susc. chk)	96.7(29)	100.0(10)a	100.0(15)a	12.7 ± 0.96 a	635.3 ± 27.5 a	92.0 ± 5.1
PI 503731	100.0(30)	70.0(7)a	85.0(14)a	13.3 ± 0.95 a	551.9 ± 54.0 abcd	80.8 ± 9.8
Standard Diet	90.0(27)	58.3(7)a	93.3(13)a	12.5 ± 0.95 a	539.6 ± 50.5 abcde	85.2 ± 9.3
Ames 10623	90.0(27)	90.0(9)a	100.0(14)a	12.9 ± 1.03 a	587.7 ± 51.2 abc	95.4 ± 4.3
PI 503727	86.7(26)	66.7(6)a	100.0(16)a	14.3 ± 0.99 a	599.0 ± 46.7 abc	96.1 ± 2.5
PI 503722	100.0(30)	70.0(7)a	80.0(16)a	12.7 ± 0.87 a	618.3 ± 32.2 ab	88.1 ± 6.3
PI 503849	93.3(28)	75.0(9)a	93.8(15)a	9.2 ± 0.95 ab	584.7 ± 36.5 abc	99.1 ± 0.9
PI 503764	96.7(28)	75.0(12)a	92.3(12)a	13.1 ± 1.07 a	562.6 ± 62.3 abcd	90.9 ± 8.3
PI 503725	100.0(30)	81.8(9)a	73.7(13)a	12.9 ± 1.01 a	522.9 ± 56.9 bcde	95.3 ± 2.3
PI 503728	96.7(29)	61.5(8)a	62.5(8)a	13.9 ± 1.11 a	633.4 ± 31.7 ab	99.9 ± 0.1
PI 503720	96.7(29)	80.0(8)a	52.6(9)b	11.6 ± 1.15 ab	467.4 ± 98.0 cde	87.3 ± 11.0
PI 503723	96.7(29)	44.4(4)a	50.0(8)b	8.7 ± 1.17 ab	537.6 ± 29.7 abcde	92.0 ± 8.0
CI31A (res. chk)	96.7(29)	40.0(4)a	47.4(9)b	7.2 ± 1.24 b	451.3 ± 60.2 de	98.0 ± 2.0
PI 485320	96.7(29)	10.0(1)b	31.6(6)b	8.6 ± 1.34 ab	405.8 ± 73.0 e	99.8 ± 0.2

[a]Survival was expressed as a percent of the total and analyzed with a chi-square test ($\alpha = 0.05$). Survival to the pupal stage was not significantly different among treatments. Survival from the pupal to the adult stage was significantly different among treatments for both males ($\alpha = 0.05$) and females ($\alpha = 0.05$). Female longevity and fecundity are expressed as the means (± SE). Longevity was analyzed by ANOVA ($\alpha = 0.05$), and pairwise comparisons within each category were made with an LSMEANS test ($\alpha = 0.05$). Fecundity was analyzed by ANOVA ($\alpha = 0.10$), and pairwise comparisons within each category were made with an LSMEANS test ($\alpha = 0.10$). Values in each category followed by the same letter indicate means that are not significantly different. There was no significant difference in fertility among females.

variability in the insects, varying year-to-year field conditions, partial chemical decomposition, metabolism, variability in the solubility of the water soluble compounds making them unavailable to the insect, or the effect as a growth inhibitor may have been influenced by another compound in the accession extract. Studies by Klun et al. (1967) of the water-soluble ECB growth inhibitor DIMBOA showed that it was metabolized from DIMBOA-glycoside, which is present in the intact leaf tissue, and that the growth inhibition caused by DIMBOA could be attenuated by addition of another plant compound, the vitamin niacin, to the diet. Similar chemical processes in Peruvian maize that eliminate or synergistically activate compounds may have mediated efficacy in our study.

The identities of compounds involved in our study have not been determined, but from previous studies of TLC extracts of Peruvian accessions, it was shown that DIMBOA and related compounds were not present in high enough quantities in leaf tissue to account for the observed ECB resistance of maize in the field (Abel et al., 1995). Bergvinson et al. (1995) found by using field-grown plants in a laboratory bioassay that third instars consumed less mature tissue than immature leaf tissue of maize synthetic BS9, despite a high concentration of DIMBOA in the immature tissue. These results led the authors to suggest that larvae are inhibited more by leaf toughness than by the presence of hydroxamic acids (DIMBOA). They noted that while DIMBOA did not seem to act as a feeding deterrent for the third instar, if a longer study encompassing the entire larval stage were done, the results might show negative effects on development and fecundity similar to those observed in the present study and those by Houseman et al. (1992) and Robinson et al. (1978). Numerous observations have demonstrated that maize plants produce and emit a complex mixture of volatile and nonvolatile compounds that are generally recognized as important cues for insect herbivores and parasites that locate and assess host suitability for feeding, refuge, and oviposition (Alborn et al., 1997; Binder and Robbins, 1997; Turlings et al., 1990; Udayagiri and Mason, 1997).

How maize natural products mediate insect behavior and physiology is becoming an intensely studied topic because of the utility of these components in integrated pest management programs (Metcalf and Metcalf, 1992). DIMBOA and related compounds have played a long and important role in the resistance of maize to ECB larvae (Klun and Robinson, 1969; Robinson et al., 1978), but apparently other factors are responsible for the antibiosis in exotic Peruvian germplasm. The possibility of additional factors contributing to ECB resistance was suggested by Bergvinson et al. (1995), who showed that maize biochemicals, such as cell wall phenolic compounds, which are responsible for leaf toughness, correlated negatively with leaf consumption by ECB third instars. The phenolics seemed to augment and help create a semiimpenetrable physical and chemical barrier. Maize silk flavonoids, such as maysin and related compounds, have been proposed as defense elements to prevent attack by larvae of the corn earworm, *Helicoverpa* *zea* (Snook et al., 1994). Maize accessions with high concentrations of these compounds were resistant to the corn earworm (Wiseman et al., 1992), and when the compounds were included separately in artificial diets fed to the larvae, all body weights declined in relation to the increasing dietary concentration of the compound (Snook et al., 1994). Like all plants, maize biosynthetic pathways produce a variety of primary and secondary natural products. Many of these, which possibly enhance multiple insect resistance, are not produced in leaf or root tissue in high enough concentrations to affect insects. As shown in our study, only a few genotypes will have high tissue levels. Evidence of the rarity of these genotypes is noted by Abel et al. (1995), who evaluated 1601 accessions from Peru for resistance to the European corn borer and found only 11 lines, representing 0.7% of the total number of evaluations, had qualities worthy of inclusion in a multiple pest resistance breeding program. These accessions express resistant traits to larval feeding and adult oviposition (Abel et al., 1995), and, as the present study shows, novel water-soluble chemical factors other than DIMBOA are produced by these exotic Peruvian accessions. These chemical traits are likely responsible for the deleterious effects on the growth, development, and survival of ECB.

The ECB-resistant Peruvian accessions are descendants of maize populations with high genetic variability, originally collected along the northwest coast of Peru and categorized by Grobman et al. (1961). They belong to four races from geographically distinct regions of Peru and illustrate the importance of searching for new genetic variants that may express qualitative and quantitative traits useful for host plant resistance in North American maize. A few maize lines have already shown resistance to multiple pests, including *O. nubilalis*, *Helicoverpa zea*, *Diatraea saccharalis*, and *Diabrotica* spp. (Davis et al., 1988; Wilson et al., 1995). Identification of chemicals responsible for resistance to ECB and other maize pests from Peruvian germplasm may have importance for maize breeders and entomologists who develop maize lines with durable plant resistance.

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